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Seroprevalence and epidemiology of African Swine Fever (ASF) in Burkina Faso

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Abstract

Background: African swine fever is a highly contagious viral disease present in most of the sub-Saharan African countries. Burkina Faso experienced its first African swine fever outbreak in 2003. The present study aims to determine the seroprevalence of African swine fever (ASF) in ASF-suspected pigs in Burkina Faso.

Result: An overall seroprevalence of 16.1% (75/466, 95% CI: 0.119-0.208) of African swine fever virus infection was observed in suspected pigs during the study period. The seroprevalence of ASF virus infection varied according to the region [Central Region (19.6%) vs Hauts-Bassins (3.3%) $p < 0.001$] and breeding system [46.7% (43/92) in modern farms vs 8.6% (32/374) in traditional farms ($p < 0.001$)]. The analysis of 230 sera randomly selected among samples collected during the study period using three ELISA kits revealed seroprevalences of 9.1% (21/230), 4.8% (11/230) and 6.5% (15/230) respectively for INGENASA® (Madrid, Spain), IDVET (Grabels, France) and SVANOVIR® ASFV-Ab (Svanova, Uppsala, Sweden) Kits.

Conclusion: The results of this study shows a high seroprevalence of African swine fever in suspected cases especially in modern farms in Burkina Faso with variations according to the regions. In the absence of vaccine against ASF infection, enhanced surveillance involving all stakeholders with awareness campaigns on biosecurity measures in farms are necessary for early detection of infection and their rapid control to prevent a possible ASF epizootic with disastrous economic consequences.

Abbreviations: ASF: African Swine Fever; CERBA: Pietro Annigoni Biomolecular Research Center; EDTA: Ethylene Diamine Tetra Acetic Acid; ELISA: Enzyme-Linked Immunosorbent Assay; FAO: Food and Agriculture Organization; LABIOGENE: Laboratory of Molecular Biology and Genetics; LNE : Laboratoire National d'Elevage; MRA : Ministère des Ressources Animales; OIE: World Organization for Animal Health; RESUREP : Réseau National de Surveillance des Maladies Animales ; WHO: World Health Organization.

Introduction

In Burkina Faso, livestock is the second largest source of export earnings after cotton and contributes more than 10 % to the formation of the Gross Domestic Product of the country [1]. Pig farming in Burkina Faso has grown significantly over the last 15 years, bringing it to the second largest pork production in West Africa after Nigeria [2-4], with a population of 2 350 430 pigs according to FAOSTAT (2016). This activity is very important in the peri-urban and urban areas but remains the most important in rural areas, from where the big cities are fed with cheap animal proteins [5]. Pigs are now permanent sources of income for the very poor, including women in rural communities [1]. The general inventory of agriculture in 2008 revealed that livestock employs 39.2 % of women. And according to FAO [2], modern pigs farming introduced by Catholic Church had put women and men into equal position. Also, in rural area, pigs are essentially managed by women who possess 60 % of the country pigs and in some regions (Centre Ouest), it can go up to 90 %. In fact, this is the only activity that women can carry out for their own [2].

Pig herds are characterized by small-scale family herds with scavenging pigs kept in the most basic traditional system and most commonly reported in urban and rural areas of developing countries [6]. In this free-range system, pigs roam freely around the house and surrounding areas and feeding in the street, from landfills or neighboring lands or from forests around villages. Little is done to provide housing for pigs [5]. Depending on the local situation, the pigs may be free during most of the year and locked up during the rainy season. They can be housed at night in a small shelter, to protect them against theft and predators. Keeping scavenging pigs requires low input use and low investment in labor, usually limited to buying food or vaccines [4].

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In this context, animals are subject to many pathogens, including African swine fever (ASF) virus. Today, the main constraint for the development of pigs remains the ASF, a highly contagious viral disease of pigs, recorded for the first time in Kenya in 1921 and widespread in most of the sub-Saharan Africa countries [7,8]. African swine fever (ASF) is a complex infectious disease of swine classified as a notifiable disease by World Organization for Animal Health. There is high risk of new introductions or reintroductions of the virus in ASF-free areas, taking into account the sylvatic cycle involving soft ticks (Ornithodorus genus) and asymptomatic wild African pigs, mainly warthogs (Phacochoerus spp) [9]. A domestic pig/tick cycle, without warthog involvement and domestic pig/pig cycle have also been described in Africa [10].

The disease is caused by a single DNA virus (*Asfivirus*), the only member of the *Asfarviridae* family [11]. There is no vaccine available against ASF and progress is hampered by lack of knowledge about the extent of virus strain diversity and viral antigens conferring specific immunity according to the viral strain in infected host [12]. Genetic characterization of ASFV clearly demonstrated the epidemiological complexity of the virus infection especially in eastern and southern Africa with 24 different genotypes described and currently at least 25 African countries reported ASFV outbreaks [13]. Eastern and Southern Africa are characterized by high genetic variability of ASFV with 22 distinct p72 genotypes in contrast with high homogeneity in West African ASFV isolates classified in a single p72 genotype I. Outside Africa, Genotype I was the only one found in Europe, America, and the Caribbean, until the recent introduction of a virus from Genotype II in Caucasus and Russia [10]. The first cases of ASF virus infections in West Africa were reported in Senegal in 1959 [14]. In recent years, ASF outbreaks have devastated swine herds in Côte d'Ivoire, Benin, Nigeria, Togo, and more recently in Gambia, Ghana and Madagascar [13,15,16].

ASFV is listed as a notifiable disease by the World Organization for Animal Health (OIE) and Burkina Faso experienced its first outbreaks in 2003 in the eastern part of the country, which shares the border with Ghana, Togo and Benin; these outbreaks were contained by slaughter and compensation of pigs [17]. Since this year several cases have been permanently notified by the national animal disease surveillance network (RESUREP) in different part of the country. Currently, there are no reports about ASF-infected wild boards in Burkina Faso.

Although ASF has a disastrous effect on the economy through the direct loss of pigs as well as market restrictions, no studies have been done to give an overview on ASF prevalence in the country even if it is known that the serotype introduced to West African countries was a serotype I virus and as they are both p72 sequences, the virus type of the Burkina Faso viruses could be determined, more studies are needed. The present study aims to conduct a survey of ASF in Burkina Faso with the detection of infection by serological tests in ASF suspected pigs according to breeding systems and province/region. This will update the ASF situation with the establishment of its epidemiological map for the application of adequate control measures to preserve the pig population and increase its productivity.

Material and method

Study sites

Burkina Faso is a Sahelian country in the heart of West Africa sharing its borders with Benin, Côte d'Ivoire, Ghana, Mali, Niger and Togo. Agriculture and livestock are an important part of the country's economy, which is subdivided into 45 provinces and 13 regions.

This study was conducted through the RESUREP; this network has 104 surveillance stations called veterinary stations, strategically located in the 45 provinces and 13 regions of the country. The sampling protocol adopted was based on information obtained from veterinary stations and alerts on pig mortality on traditional and modern farms. The geographical distribution of the different sampling sites in the regions of Burkina Faso between 2014 and 2016.

Study design

A cross-sectional study to determine the seroprevalence of ASF virus infection in suspected cases or pigs with clinical signs suggestive of a possible infection with the ASF virus in modern and traditional farms through different province/region was conducted between 2014 and 2016. During the study period, the veterinary posts of RESUREP notified through their monthly reports suspected cases of ASF in 24 localities in 16 provinces of 11 regions of the country. A total of 416 sera samples were collected from different farms within province/region and sent to the laboratory for further analysis.

In addition to the RESUREP notification, 11 alerts on pig mortality in modern farms have been notified in peri-urban areas of Ouagadougou and Bobo-Dioulasso between February 2014 and November 2015. Laboratory technicians and an epidemiologist investigated these alerts and collected biological samples including 50 sera. The date of alerts during the study period are presented in Table 1.

Traditional farms

These are very small family farms of two to ten pigs, the vast majority of which are stray (90 %, called "pig runners"). They value domestic waste, dolo[1] dill (by-product of millet beer) and all other waste and crop residues they find by walking around villages and fields after harvest.

They are extensive farms with a minimum or no infrastructure investment in terms of buildings, equipment or livestock facilities. This type of farm uses local breeds of pigs and is more important in rural areas thus making it possible to supply cities and the countryside with animal proteins at a lower cost.

Modern farms

These are improved and intensive or semi-intensive holdings with infrastructure investment. These farms are located in urban and peri-urban areas, some of which use imported exotic pig breeds. There is only one local breed in Burkina Faso called “porc coureur”. The mains exotic breeds used by modern farms to enhance their productivity are “Korhogo” from Ivoiry coast and “Large white” from England. We can

[1]Ancestral beer obtained by the fermentation of red sorghum or germinated millet and cooked in water, very widespread in Sahelian Africa.

Table 1. Date of alerts in province/region during the study period

Region	*Province*	*Locality*	*Date of alert*
Centre	**Kadiogo**	Koubri	February 13th, 2014 August 12ve, 2014 August 26th, 2015
		Saaba	June 12ve, 2014 June 1st, 2015
		Nioko II	June 2nd, 2015
		Wapassi/ Ouagadougou	November 24th, 2015
Centre-Sud	**Bazega**	Sapone	February 10th, 2015
Hauts-Bassins	**Bobo-Dioulasso**	Banakeledaga	September 25th, 2014
		Bobo-Dioulasso	October 7th and 20th, 2015

also find in some farms "Hampshire" from England "Land race" from Danish, "Duroc" from America, and "Pietrain" from Belgium. Animals confinement at high stocking density is observed in modern farms with issues including efficiency of food production in biosecurity conditions. These farms are increasingly specialized with an organization of actors in the pork industry.

Sample collection

Samples were collected from February 2014 to February 2016. Veterinary post officers in charge of surveillance and sample collection performed blood sampling from the jugular vein primarily and secondarily from the saphenous and auricular veins in both free anticoagulant vacutainer and EDTA tubes and then used respectively for subsequent sera and plasma collection. The sera collected in Eppendorf microtubes were centrifuged at 1500 rpm for 5 minutes to remove traces of red blood cells and impurities and then stored at -20 °C until used for Enzyme-Linked Immunosorbent Assay (ELISA). Samples were collected upon suspicions or alert investigation and multiples samples (sera and or organs) were collected when necropsy were possible. Number of sera collected in a single farm varies from 2 to 10 according to the pig population in the farm and considering that sometimes majority of pigs are dead or quickly sold out.

In the case of suspicion, samples are often collected from 2 to 5 different farms which share the same environment in a free-range system.

During the study period some locality were visited twice (Saaba, Dano, Nioko and Saponé) or three times (Bobo-Dioulasso, Koubri), we consider those locations as an ASF persistent area.

Laboratory analysis

The ELISA tests were performed using the ID Screen® African Swine Fever Indirect antibody ELISA Kit (IDVET, Grabels, France) according to the protocol provided by the manufacturer. In addition to this, the IDVET Kit results were compared with those obtained using two other ELISA commercial kits namely INGENASA® (Madrid, Spain) and SVANOVIR® ASFV-Ab (Svanova, Uppsala, Sweden) on a sample of 230 sera randomly selected from sera samples collected between 2014 and 2016.

Statistical analysis

The data was recorded on Microsoft Excel 2013 and analyzed using the Epi Info 7.0 software. The Chi square test was used for comparisons with a significance threshold set at $p < 0.05$. The 95% confidence intervals for seroprevalences were determined using the R Software through the "binom.confint" function of the "library (binom)" and the "exact" method.

Result

Seroprevalence of ASF in suspected pigs

In this study, an overall seroprevalence of approximately 16.1% (75/466, 95% CI: 0.129 - 0.198) of ASF virus infection was recorded in all suspected cases reported during the study period (Table 2). In a sample of 416 suspected cases from RESUREP notifications, a prevalence of 15.6% (65/416, 95% CI: 0.123 - 0.195) of pigs seropositive for ASF virus infection was observed against 20.0% (10/50) of positive cases recorded following alerts on pig mortality in modern farms in peri-urban areas of Ouagadougou and Bobo-Dioulasso.

The seroprevalence of ASF infection among the reported suspected cases varied according to the region with the highest frequencies recorded in Sanguié (46,1%; 12/26) and Seno (50.0%; 6/12) and Poni (60.0%; 3/5) provinces. The prevalence of infection was significantly higher in the Central region compared to the Hauts-Bassins region (22.6 [30/133] versus 3.3 [3/92], $p < 0.001$). In this study population, 19.7% (92/466) of suspected cases were from modern farms versus 80.3% (374/466) from traditional farms. The prevalence of ASF virus infection was significantly higher (43/92) among suspected cases from modern farms compared to those (32/374) from traditional farms (46.7% vs 8.6%; $p < 0.001$).

Comparative tests of ELISA kits

A comparative test of the IDVET kit (Grabels, France) used for the different ELISA analyzes in this study with two other ELISA kits namely

Table 2. Prevalence of African swine fever in suspected cases from RESUREP and Alerts between 2014 and 2016

Provinces/Regions		Samples					ELISA results		
		RESUREP (N)		Alerts (N)		Total	Neg.	Pos.	Seroprevalence
Provinces	Regions	MF	TF	MF	TF	All	n	n	%
Bazega	Centre-Sud	0	6	**2**	2	10	8	**2***	20.0
Houet	Hauts-Bassins	0	86	0	6	92	89	**3**	**3.3**
Bougouriba	Sud-Ouest	0	31	-	-	31	31	0	0.0
Boulkiemdé	Centre-Ouest	0	15	-	-	15	15	0	0.0
Ioba	Sud-Ouest	0	15	-	-	15	12	3	20.0
Kadiogo	Centre	**38**	**55**	**21**	19	133	103	**30****	**22.6**
Kompienga	Est	0	28	-	-	28	23	**5**	**17.9**
Koulpelogho	Centre-Est	0	17	-	-	17	15	**2**	**11.8**
Lorum	Nord	0	5	-	-	5	5	0	0.0
Namentenga	Centre- Nord	0	18	-	-	18	15	3	16.7
Oubritenga	Plateau-Central	0	13	-	-	13	13	0	0.0
Poni	Sud-Ouest	5	0	-	-	5	2	**3**	**60.0**
Sanguié	Centre-Ouest	26	0	-	-	26	14	**12**	**46.1**
Seno	Sahel	0	12	-	-	12	6	**6**	**50.0**
Topoa	Est	0	23	-	-	23	17	**6**	**26.1**
Ziro	Centre-Ouest	0	23	-	-	23	23	0	0.0
Total		**69**	**347**	**23**	**27**	**466**	**391**	**75**	**16.1**

*The two positive cases were from modern farms through alerts; **26 positive cases (18/38 through RESUREP and 8/21 through alerts) were from modern farms; **MF** = Modern Farm**; TF** = Traditional Farm. **Neg.** = Negative, **Pos.** = Positive.

INGENASA® (Madrid, Spain) and SVANOVIR® ASFV-Ab (Svanova, Uppsala, Sweden) was carried out. The analysis of 230 sera randomly selected using these three kits revealed seroprevalences of 9.1% (21/230), 4.8% (11/230) and 6.5% (15/230) respectively for INGENASA® (Madrid, Spain), IDVET (Grabels, France) and SVANOVIR® ASFV-Ab (Svanova, Uppsala, Sweden) Kits.

Although a relatively higher seroprevalence was obtained by the INGENASA® Kit, no statistically significant difference was observed between seroprevalence obtained with these three kits (INGENASA® vs IDVET, p = 0.067, INGENASA® vs SVANOVIR® ASFV -Ab, p = 0.298). The results obtained by the different Kits are shown in Table 3.

Discussion

African swine fever is a highly contagious viral disease with 100% morbidity and mortality rates between 0 and 100% depending on the factors related to the virus, the host and the route of exposure.

The disease causes serious socio-economic repercussions through the direct loss of animals and market restrictions. The objective of this study was to determine the seroprevalence of ASF in suspected cases through RESUREP and pig mortality alerts in modern farms.

An overall seroprevalence of 16.1% (75/466) of ASF virus infection was recorded during the study period with 46.7% (43/92) of infection cases in the modern farms compared to 11.7% (32/374) in traditional farms. The high overall seroprevalence of the present study is due to the fact that the investigations were carried out directly in pigs with clinical signs suggestive of a possible infection with the ASF virus while the significantly high frequency of infection in modern farms suggests a low level of biosecurity [17]. Breeding of pigs in a closed environment, required by the modern breeding system with a higher number of animals are also factors that can explain this high prevalence. An earlier study in Kenya also reported variations in prevalence according to livestock system [18]. The prevalence of 16.1% observed in this study is similar to the 16.9% (126/747) reported in 2006 in Senegal [14]. However, it is lower than the 53.0% prevalence observed in slaughter pigs in Kenya in 2013 [19]. A recent study in Côte d'Ivoire confirmed the presence of ASF virus infection in swine samples from Burkina Faso by PCR method [20]. Indeed, pig straying in the traditional rearing system, soil condition of the habitat, feeding source, lack of preventive care and low level of biosecurity of the farms are the main risk factors of introduction and spread of ASF [4,20,21]. Even though modern farms are more organized and invest in modernizing pig farming, there is an insufficient application of biosecurity measures. We can note the insufficient feeds and water supply system. There is no specific transportation system for feeds to the farm and animals to slaughterhouses. Access to farm is often easy and employees sometimes misuse or avoid the less biosecurity measures put in place.

They are also big consumers of pig's meat sold in the surrounding of farm they are in charge of almost modern farms sell their animals to pork-butchers and animals are usually slaughtered in slaughterhouses and carcasses undergo veterinary inspection and transported by refrigerated vehicle. In addition, pig meats are widely consumed in rural and urban areas in Burkina Faso. Pig comes from across the country, transported with inadequate vehicle sometimes mixed with other animals or humans. They are sold in improvised markets to roasters and butchers, often slaughter out of slaughterhouses or clandestinely with lack of hygiene and without veterinary inspection [16]. Clandestinely slaughtering of pigs in open area mainly in the village lead to a potential dissemination of diseases such as ASF among domestic pigs and wild boar during the hunting season; moreover, hunted wild board are prepared and the meats consumed in the village. Domestic pigs can also consume by-products from wild boar carcasses. This situation enhances the risk of ASF circulation among the two populations [2] even if no investigation is done in the wild boar population.

In the present study, the seroprevalence of ASF virus infection varied according to the province or region during the study period. Indeed, a significantly higher prevalence (22.6%) was recorded in the Central region compared to the Hauts-Bassins region (3.3%) during

Table 3. Comparative test results of three ELISA kits

Location	Number of sera	Results of analysis					
		INGENASA Kit		IDVET Kit		SVANOVIR ASF Ab Kit	
		Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Nakamtenga	13	**0**	13	**0**	13	**0**	13
Bobo Dioulasso	29	**4**	25	**3**	26	**4**	25
Koubri	8	**5**	3	**2**	6	**1**	07
Dano	15	**0**	15	**0**	15	**0**	15
Kompienga	13	**0**	13	**0**	13	**0**	14
Kindi	14	**1**	13	**0**	14	**0**	14
Bittou	20	**3**	17	**1**	19	**0**	20
Gampéla	12	**0**	12	**0**	12	**0**	12
Boulsa	10	**0**	10	**0**	10	**0**	10
Réo	10	**0**	10	**0**	10	**1**	09
Banakeledaga	30	**0**	30	**0**	30	**0**	30
Saponé	3	**0**	3	**0**	3	**0**	03
Saaba	2	**0**	2	**0**	2	**0**	02
Dori	7	**1**	6	**0**	6	**2**	05*
Sabou	1	**0**	1	**0**	1	**0**	01
Wapassi	10	**0**	10	**0**	10	**1**	09
Tikaré	5	**0**	5	**0**	5	**0**	05
Kantchari	23	**4**	19	**3**	20	**5**	18
Yamtenga	5	**3**	2	**2**	3	**1**	04
Total	230	21	209	11	218	15	215

*1 result was inconclusive in samples from Dori; **Neg.** = Negative, **Pos.** = Positive.

the study period. In their study in Senegal, Etter *et al.* (2006) reported prevalence of 13.3%, 7.8%, and 22.1% respectively in the Fatick, Kolda and Ziguinchor regions. These variations between different regions indicate the complexity of the epidemiology of ASF [13,22-24].

The present study population consisted of over 80.3% (374/466) pigs from traditional farms versus 19.7% (92/466) from modern farms. These observations confirm the predominance of the traditional breeding system in pig production in Burkina Faso. In fact, according to FAO estimates, traditional livestock breeding with 90% of livestock produce 85% of pork in Burkina Faso [2].

In this study, seroprevalences of 9.1%, 4.8% and 6.5% were obtained respectively with INGENASA®, IDVET and SVANOVIR® ASFV-Ab Kits. These are the three main ELISA commercial kits available for the detection of anti-ASF antibodies including INGEZIM PPA COMPAC, K3 of the INGENASA company, which is the most widely used in European countries [25]. The techniques currently used for diagnosis of ASF provide reliable results in any epidemiological situation. However, the diagnosis of ASF virus infection is complex and not always easy because of the wide range of clinical forms. Indeed, the variation in the results depends mainly on the sensitivity and the specificity of each test [26]. Gallardo *et al.* [25] showed that ELISA tests were unable to detect infected pigs with antibody titers below 1/640 for the INGENASA-ELISA kit and below 1/5210 for the IDVET and SVANOVA tests. The authors also reported a high sensitivity and low specificity of INGENASA tests in the detection of anti-ASF antibodies compared to IDVET and SVANOVA tests [25]. The choice of the ELISA Kit may therefore lead to overestimation or underestimation of the seroprevalence of ASF virus infection and sometimes requiring direct detection of the virus in blood by Polymerase Chain Reaction (PCR). Indeed, Okoth *et al.* [18] reported a prevalence of 28% of pigs positive for ASF virus infection by PCR with no detectable clinical signs, all of which were seronegative in the ELISA test recommended by the OIE . The seroprevalence estimate of the present study was performed using the IDVET kit, which has the lowest prevalence (4.8% vs 6.5% and 9.1%) of the three kits tested. This suggests a probably higher seroprevalence in our study population. This observation requires confirmation using a more specific and sensitive method, namely PCR for direct detection of viral DNA in suspected pigs.

Conclusion

The results of this study show a high seroprevalence of African swine fever in Burkina Faso with variations depending on the region and the breeding system.

These results support the fact that veterinary services consider the disease as enzootic in the country. In the absence of vaccine against the disease, enhanced surveillance involving all stakeholders with awareness campaigns on biosecurity measures in farms are necessary for early detection of infection cases and their rapid control to prevent a possible epizootic of the ASF with disastrous economic consequences.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interests

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Authors' contribution

GLM and **JS*** conceived and designed the study. **GLM, JS** and **EK** were involved in data generation, collection and assembly. **GLM** and **AKO** were involved in data analysis and interpretation. **GLM**, **AKO**, **DO-Y**, **JS**, **EK**, **SM**, **AO, VO**, **HU** and **JS*** were involved with drafting or revising the manuscript. **GLM**, **HU** and **JS*** provided administrative, technical and material support. Supervision of the study was made by **GLM**, **AKO** and **JS***. All authors critically revised and approved the final version of this publication.

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